GLYCINE CONJUGATION: A METABOLIC PATHWAY OF *N*-ALKYL SUBSTITUTED MONO-CYCLOPARAFFINS

J. TULLIEZ and J. C. PELERAN

Laboratoire de Recherches sur les Additifs Alimentaires, INRA, 180, Chemin de Tournefeuille, 31300 Toulouse, France

Received 26 April 1977

1. Introduction

In a previous work [1], it has been stated that rats were able to ω-oxidize the *n*-alkyl substituent of dodecylcyclohexane, then to β-oxidize the resulting cyclohexyldodecanoic acid. The final product of this process may be cyclohexylacetic acid. Little is known of the metabolic transformations of simple alicyclic hydrocarbons [2,3] and no data is available concerning the fate of naphtenic hydrocarbons with *n*-alkyl side chains. Our investigation of the metabolism of dodecylcyclohexane attempts to identify the urinary excretion products.

It is well known that many aromatic acids are conjugated with amino acids before elimination in urine. Glycine is the most common of the endogenous amines to conjugate organic acids; thus in the rat, benzoic and phenylacetic acids are excreted as hippuric and phenylaceturic acids. As far as cyclohexyl-acids are concerned, it has been established [4–7] that aromatization of cyclohexane carboxylic acid only occurs to a large extent in the dog and the rabbit and not in the rat, whereas ring breakdown seems to occur with cyclohexylacetic acid [6]. Therefore our research was conducted towards the identification of the glycine conjugate.

2. Materials and methods

Male Wistar rats weighing about 200 g were held in metabolism cages and maintained on a semi-synthetic diet; urine was collected two days before, and two days after, a single dose (100 mg) of dodecylcyclohexane (Koch Light Laboratories). After acidifying to pH 2, urine was extracted twice with redistilled ethyl acetate. The combined extracts were dried over sodium sulfate, evaporated to dryness, then esterified with ethanolic HCl. The ethyl esters obtained were analysed by gas–liquid chromatography (GLC) on a cyanopropyl–phenyl silicone column (3% OV-225 on 100–120 mesh chromosorb W AW DMCS). Retention times were compared to those of ethyl esters of phenyl and cyclohexyl acids conjugated with glycine. Hippuric acid was from Fluka and phenylaceturic acid synthesized by reaction of glycine ethyl ester with phenylacetyl chloride. The latter was prepared by reaction of $SOCl_2$ with phenylacetic acid (Fluka). Glycine conjugates of cyclohexane carboxylic and cyclohexylacetic acids were obtained by catalytic hydrogenation of the corresponding aromatic compounds.

Urinary extracts in the form of ethyl esters were treated in different ways before GLC analysis. Reduction by hydrogen was performed using PtO_2 in methanol and bromination with Br_2 in chloroform. Acetylation was carried out with acetic anhydride. Confirmation of molecular structure of unknown compounds isolated from urines was accomplished by gas chromatographic–mass spectroscopic (GC–MS) analysis.

3. Results and discussion

GLC analysis of esterified urinary extract (fig.1) shows a peak, that the reference extract does not exhibit; its retention time corresponds to that of

North-Holland Publishing Company – Amsterdam

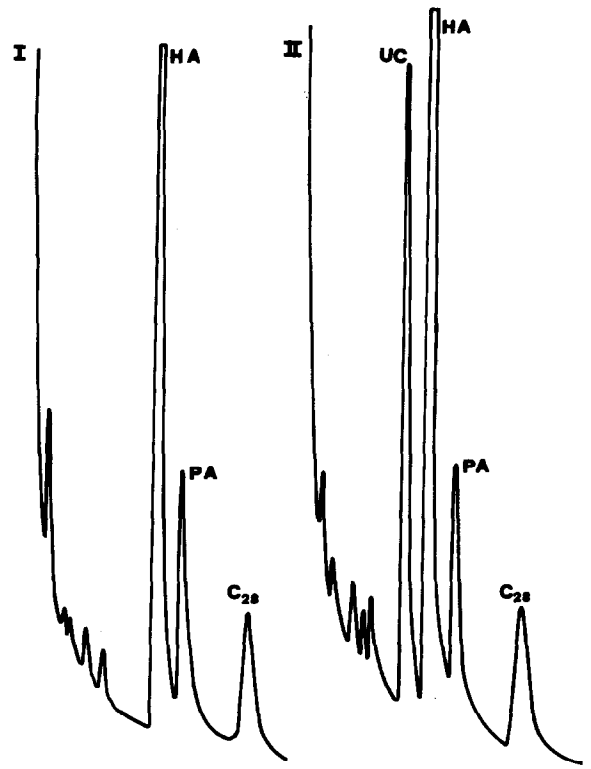

Fig.1. GLC analysis of urinary extracts in the form of ethyl esters. (I) Control urines. (II) Experimental urines. (HA) Hippuric acid, (PA) Phenylaceturic acid, (C_{28}) octacosane internal standard, (UC) unknown compound.

cyclohexylaceturic acid. GLC analysis of the hydrogenated extract, reveals the reduction of hippuric acid to cyclohexane–carboxyl-glycine, whereas retention time of the unknown compound (UC) is not modified. When brominated, the extract reveals no peak in the previous chromatographic conditions, indicating that the excretion product of dodecylcyclohexane is unsaturated. Acetylation of the esterified urinary extract does not modify in any way the chromatogram, which excludes the hydroxylation of cyclohexane ring.

The mass spectrum (fig.2) of UC reveals a molecular ion at m/e 225 that differs only in two mass units from that of cyclohexylaceturic acid. The presence of a peak at m/e 81 associated with peaks at m/e 67 and m/e 53 suggests a cyclohexene structure which would explain the mass difference observed. Moreover, the mass spectrum of hydrogenated UC was identical to that of cyclohexylaceturic acid. Thus UC may be a cyclohexenylacetic acid conjugated with glycine. All the expected major ions for such a compound are present in the spectrum. Amide breakdown leads to peaks at m/e 122 and m/e 104 if referred to a double hydrogen rearrangement on the nitrogen [8]. The peak at m/e 94 can be accounted for by a retro Diels-Alder type cleavage, typical of unsaturated cyclic compounds [9], whereas the metastable peak at m/e 66.4 would

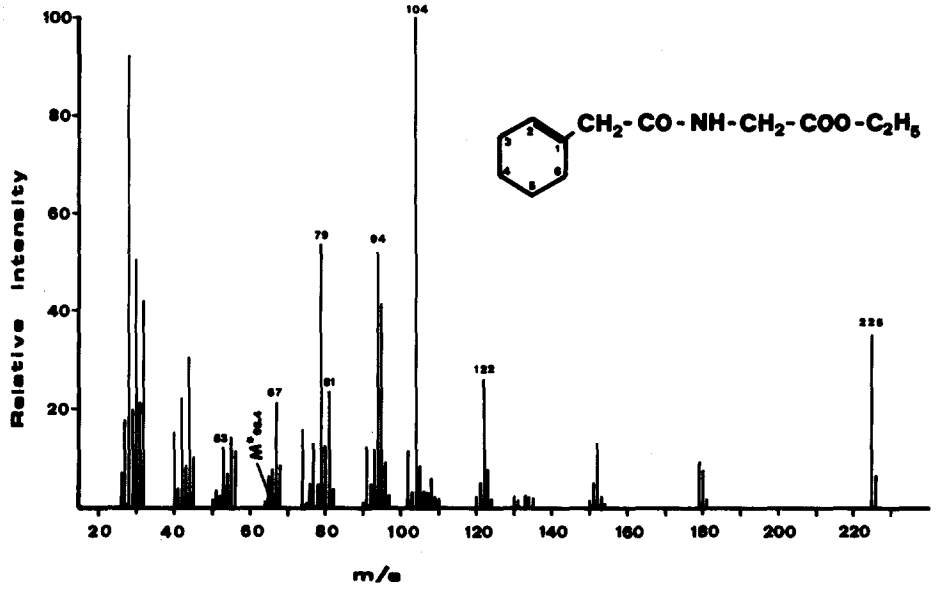

Fig.2. Mass spectrum of the unknown compound (UC) in the form of ethyl ester.

account for the m/e 94 to m/e 79 transition. The presence of the peak at m/e 94 eliminates the double bond location in position 3 according to the retro Diels-Alder cleavage. The exact position of the double bond is under investigation.

This work shows that dodecylcyclohexane leads to the elimination of a compound in the form of a glycine conjugate. Cyclohexenylaceturic acid results from cyclohexylacetic acid, produced by the oxidation of the cycloparaffin. To our knowledge this type of conjugate has never been observed and would complete the results of Bernhard [4,5] and Dickens [6] who established that cyclohexane compounds containing odd numbered fatty acid side chains were aromatized then conjugated with glycine, whereas they supposed that ring breakdown occurred with even numbered carbon side chains.

Acknowledgements

This work was supported by research grant 75-136 awarded by the Ministère de la Qualité de la Vie et de l'Environment (France). We are grateful to M. Rigaud and P. Dubois (INRA, Dijon, France) for GC–MS analysis and to G. Delous for technical assistance.

References

[1] Tulliez, J. and Peleran, J. C. (1977) FEBS Lett. 75, 120–123.
[2] Elliott, T. H., Parke, D. V. and Williams, R. T. (1959) Biochem. J. 72, 193–200.
[3] Elliott, T. H., Tao, R. C. C. and Williams, R. T. (1965) Biochem. J. 95, 70–76.
[4] Bernhard, K. (1937) Hoppe-Seyl. Z. 248, 256.
[5] Bernhard, K. (1938) Hoppe-Seyl. Z. 256, 49–58.
[6] Dickens, F. (1950) Biochem. Soc. Symp. 5, 66–84.
[7] Beer, C. T., Dickens, F. and Pearson, J. (1951) Biochem. J. 48, 222–237.
[8] Mc Lafferty, F. W. and Hamming, M. C. (1958) Chem. Ind. 1366–1367.
[9] Biemann, K. (1962) in: Mass Spectrometry: organic chemical applications, Mc Graw-Hill, NY.